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Heat Inactivation of Restriction Endonucleases

Heat inactivation is a convenient method for stopping a restriction endonuclease reaction. Incubation for 20 minutes inactivates the majority of restriction endonucleases that have an optimal incubation temperature of 37°C. Enzymes that cannot be inactivated at 65°C can often be inactivated by incubation at 80°C for 20 minutes. The table below indicates whether or not an enzyme can be heat inactivated and the temperature needed to do so.

Heat inactivation was performed as follows to approximate a typical experiment. A 50 µl reaction containing the appropriate NEBuffer, 0.5 µg of calf thymus DNA, and 5 or 10 µl of restriction enzyme (final concentration) was incubated at 37°C for 60 minutes and then at 65°C or 80°C for 20 minutes. Substrate DNA (usually lambda) was added to the reaction mixture and incubated at the optimal temperature of the enzyme for 60 minutes. Any digestion (complete or partial) of the substrate DNA during the second incubation, as seen by agarose gel electrophoresis, was interpreted as incomplete heat inactivation.

A | B | C | D | E | F | H | I | K | M | N | P | R | S | T | X | Z |

Enzyme	Heat Inactivation Temperature	Heat Inactivation Time
AatII	65°C	20 min
Acc65I	65°C	20 min
AccI	80°C	20 min
AcII	65°C	20 min
AcII	No	--
AcuI	65°C	20 min
AfeI	65°C	20 min
AflII	65°C	20 min
AflIII	80°C	20 min
AgeI	65°C	20 min
AhdI	65°C	20 min
AleI	65°C	20 min
AluI	65°C	20 min
AlwI	65°C	20 min
AlwNI	65°C	20 min
ApaI	65°C	20 min
ApaLI	No	--
ApeKI	No	--
ApoI	80°C	20 min
AscI	65°C	20 min
AseI	65°C	20 min
AsiSI	80°C	20 min
AvaI	80°C	20 min
AvaII	65°C	20 min

AvrII	No	--
BaeI <top>	65°C	20 min
BamHI	No	--
BanI	65°C	20 min
BanII	65°C	20 min
BbsI	65°C	20 min
BbvCI	80°C	20 min
BbvI	65°C	20 min
BccI	65°C	20 min
BceAI	65°C	20 min
BcgI	65°C	20 min
BciVI	65°C	20 min
BclI	No	--
BfaI	80°C	20 min
BfuAI	65°C	20 min
BglI	65°C	20 min
BglII	No	--
BlpI	No	--
Bme1580I	80°C	20 min
BmgBI	65°C	20 min
BmrI	65°C	20 min
BmtI	65°C	20 min
BpmI	65°C	20 min
Bpu10I	80°C	20 min
BpuEI	65°C	20 min
BsaAI	80°C	20 min
BsaBI	80°C	20 min
BsaHI	80°C	20 min
BsaI	65°C	20 min
BsaJI	80°C	20 min
BsaWI	80°C	20 min
BsaXI	No	--
BseRI	65°C	20 min
BseYI	65°C	20 min
BsgI	65°C	20 min
BsiEI	80°C	20 min
BsiHKAI	80°C	20 min
BsiWI	80°C	20 min
BsII	80°C	20 min
BsmAI	80°C	20 min
BsmBI	80°C	20 min
BsmFI	80°C	20 min
BsmI	80°C	20 min

BsoBI	No	--
Bsp1286I	65°C	20 min
BspCNI	80°C	20 min
BspDI	65°C	20 min
BspEI	80°C	20 min
BspHI	65°C	20 min
BspMI	65°C	20 min
BspQI	80°C	20 min
BsrBI	80°C	20 min
BsrDI	80°C	20 min
BsrFI	No	--
BsrGI	80°C	20 min
BsrI	80°C	20 min
BssHII	80°C	20 min
BssKI	80°C	20 min
BssSI	80°C	20 min
BstAPI	80°C	20 min
BstBI	No	--
BstEII	No	--
BstNI	No	--
BstUI	No	--
BstXI	65°C	20 min
BstYI	80°C	20 min
BstZ17I	No	--
Bsu36I	80°C	20 min
BtgI	80°C	20 min
BtgZI	80°C	20 min
BtsCI	No	--
BtsI	80°C	20 min
Cac8I <top>	65°C	20 min
ClaI	65°C	20 min
CspCI	No	--
CviAII	65°C	20 min
CviKI-1	80°C	20 min
DdeI <top>	65°C	20 min
DpnI	80°C	20 min
DpnII	65°C	20 min
DraI	65°C	20 min
DraIII	65°C	20 min
DrdI	65°C	20 min
EaeI <top>	65°C	20 min
EagI	65°C	20 min
EarI	65°C	20 min

EciI	65°C	20 min
EcoNI	65°C	20 min
EcoO109I	65°C	20 min
EcoP15I	65°C	20 min
EcoRI	65°C	20 min
EcoRV	80°C	20 min
FatI <top>	65°C	20 min
FauI	65°C	20 min
Fnu4HI	65°C	20 min
FokI	65°C	20 min
FseI	65°C	20 min
FspI	65°C	20 min
HaeII <top>	80°C	20 min
HaeIII	80°C	20 min
HgaI	65°C	20 min
HhaI	65°C	20 min
HincII	65°C	20 min
HindIII	65°C	20 min
HinfI	80°C	20 min
HinP1I	65°C	20 min
HpaI	No	--
HpaII	65°C	20 min
HphI	65°C	20 min
Hpy188I	65°C	20 min
Hpy188III	65°C	20 min
Hpy99I	65°C	20 min
HpyCH4III	80°C	20 min
HpyCH4IV	65°C	20 min
HpyCH4V	65°C	20 min
I-CeuI <top>	65°C	20 min
I-SceI	65°C	20 min
KasI <top>	65°C	20 min
KpnI	No	--
MboI <top>	65°C	20 min
MboII	65°C	20 min
McrBC	65°C	20 min
MfeI	65°C	20 min
MluI	65°C	20 min
MlyI	65°C	20 min
MmeI	80°C	20 min
MnII	65°C	20 min
MscI	65°C	20 min
MseI	65°C	20 min

MslI	65°C	20 min
MspA1I	65°C	20 min
MspI	65°C	20 min
MwoI	No	--
NaeI <top>	65°C	20 min
NarI	65°C	20 min
Nb.BbvCI	80°C	20 min
Nb.BsmI	80°C	20 min
Nb.BsrDI	No	--
Nb.BtsI	80°C	20 min
NciI	No	--
NcoI	65°C	20 min
NdeI	65°C	20 min
NgoMIV	80°C	20 min
NheI	65°C	20 min
NlaIII	65°C	20 min
NlaIV	65°C	20 min
NotI	65°C	20 min
NruI	65°C	20 min
NsiI	80°C	20 min
NspI	65°C	20 min
Nt.AlwI	80°C	20 min
Nt.BbvCI	80°C	20 min
Nt.BstNBI	80°C	20 min
Nt.CviPII	65°C	20 min
PacI <top>	65°C	20 min
PaeR7I	No	--
PciI	80°C	20 min
PfIFI	65°C	20 min
PfIMI	65°C	20 min
PhoI	No	--
PI-PspI	No	--
PI-SceI	65°C	20 min
PleI	65°C	20 min
PmeI	65°C	20 min
PmlI	65°C	20 min
PpuMI	No	--
PshAI	65°C	20 min
PsiI	65°C	20 min
PspGI	No	--
PspOMI	65°C	20 min
PspXI	80°C	20 min
PstI	80°C	20 min

PvuI	80°C	20 min
PvuII	No	--
RsaI <top>	65°C	20 min
RsrII	65°C	20 min
SacI <top>	65°C	20 min
SacII	65°C	20 min
SalI	65°C	20 min
SapI	65°C	20 min
Sau3AI	65°C	20 min
Sau96I	80°C	20 min
SbfI	80°C	20 min
ScaI	80°C	20 min
ScrFI	65°C	20 min
SexAI	65°C	20 min
SfaNI	65°C	20 min
SfcI	65°C	20 min
SfiI	No	--
SfoI	65°C	20 min
SgrAI	65°C	20 min
SmaI	65°C	20 min
SmlI	No	--
SnaBI	80°C	20 min
SpeI	65°C	20 min
SphI	65°C	20 min
SspI	65°C	20 min
StuI	65°C	20 min
StyD4I	65°C	20 min
StyI	65°C	20 min
SwaI	65°C	20 min
Taq ^q I <top>	80°C	20 min
TfiI	No	--
TliI	No	--
TseI	No	--
Tsp45I	No	--
Tsp509I	No	--
TspMI	No	--
TspRI	No	--
Tth111I	No	--
XbaI <top>	65°C	20 min
XcmI	65°C	20 min
XhoI	65°C	20 min
XmaI	65°C	20 min
XmnI	65°C	20 min

ZraI <top>	80°C	20 min
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